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DOI:

[10.1038/s41380-018-0335-7](https://doi.org/10.1038/s41380-018-0335-7)

Document Version

Peer reviewed version

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Citation for published version (APA):

Legge, S. E., Pardiñas, A. F., Helthuis, M., Jansen, J. A., Jollie, K., Knapper, S., MacCabe, J. H., Rujescu, D., Collier, D. A., O'Donovan, M. C., Owen, M. J., & Walters, J. T. R. (2019). A genome-wide association study in individuals of African ancestry reveals the importance of the Duffy-null genotype in the assessment of clozapine-related neutropenia. *Molecular Psychiatry*, 24(3), 328-337. <https://doi.org/10.1038/s41380-018-0335-7>

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Duffy-null genotype in clozapine-associated neutropenia

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Abstract

Background

Individuals of African ancestry in the US and Europe are at increased risk of developing schizophrenia and have poorer clinical outcomes. The antipsychotic clozapine, the only licensed medication for treatment-resistant schizophrenia, is under-prescribed and has high rates of discontinuation in individuals of African ancestry, due in part to increased rates of neutropenia. The genetic basis of lower neutrophil levels in those of African ancestry has not previously been investigated in the context of clozapine treatment.

Methods

We sought to identify risk alleles in the first genome-wide association study of neutrophil levels during clozapine treatment, in 552 individuals with treatment-resistant schizophrenia and robustly inferred African genetic ancestry.

Results

Two genome-wide significant loci were associated with low neutrophil counts during clozapine treatment. The most significantly associated locus was driven by rs2814778 ($\beta = -0.9$, $p = 4.21 \times 10^{-21}$), a known regulatory variant in the Atypical Chemokine Receptor 1 (*ACKR1*) gene. Individuals homozygous for the C allele at rs2814778, known as the Duffy-null genotype, were significantly more likely to develop neutropenia and have to stop clozapine treatment ($OR = 20.4$, $p = 3.44 \times 10^{-7}$).

Conclusions

Low neutrophil counts in individuals of African ancestry do not necessarily indicate an adverse drug reaction to clozapine, but can reflect benign ethnic neutropenia (BEN) due to the Duffy-null genotype. Thus, the rs2814778 genotype could be used as a pharmacogenetic test to revise safety-monitoring criteria for clozapine and address its under-prescription. This represents an important advance in personalised medicine in psychiatry with the potential to improve access to treatment for those of African ancestry with schizophrenia.

Introduction

The incidence of psychotic disorders is increased in migrant populations^{1,2}. In particular, individuals of African and African-Caribbean ethnicity in the UK and African American ethnicity in the US are at especially high risk of developing schizophrenia^{3,4}. The exact causes of this are unknown, but are at least in part due to factors associated with being a member of a disadvantaged minority, such as socioeconomic stress and isolation^{5,6}. Those of African and African-Caribbean ancestry have also been consistently demonstrated to have poorer clinical outcomes including higher rates of inpatient admissions involving the police or compulsion^{7,8}. Furthermore, a recent study examining cases of first-episode psychosis in the UK found that in comparison with White British patients, Black African and Black African-Caribbean patients had a worse long-term clinical course with lower rates of recovery⁸.

The antipsychotic clozapine is the only licenced treatment for those with schizophrenia who fail to respond to other antipsychotics (termed treatment-resistant schizophrenia)^{9,10}. While clozapine has been robustly demonstrated to reduce the risk of rehospitalisation and to be associated with better symptomatic and functional outcomes¹¹, it is widely under-prescribed. Clozapine is particularly under-utilised in those of African American ethnicity in the US and Black African or African-Caribbean ethnicity in the UK¹²⁻¹⁶, populations that have also been consistently associated with a higher rate of clozapine discontinuation^{14,15,17-19}. This is at least partly due to increased rates of neutropenia in those of African ancestry whilst taking clozapine^{19,20}. Neutropenia is defined as an absolute neutrophil count less than 1500 cells/mm³, while agranulocytosis is diagnosed if the neutrophil count is below 500 cells/mm³ and is a severe condition that can endanger life. Clozapine treatment increases the cumulative risks of neutropenia (2.9%) and agranulocytosis (0.8%), which has led many countries to introduce regulatory systems for frequent blood monitoring whilst on the medication²¹.

Recent evidence has indicated that genetics plays a role in susceptibility to neutropenia on clozapine. Genetic association studies have implicated *HLA-DQB1* and *HLA-B* variants^{22,23}, and rs149104283, a SNP intronic to transcripts of hepatic transporter genes *SLCO1B3* and *SLCO1B7*²³ in individuals of European ancestry. Reflecting a general dearth of clinical and genetic research in non-European populations, all studies to date investigating clozapine-associated neutropenia have focused exclusively on individuals

of European ancestry, limiting the generalizability of findings. This is particularly relevant to those of African ancestry who are known to have lower baseline neutrophil counts on average compared to those from other populations²⁴; this does not seem to impart an increased susceptibility to infection²⁴ but nonetheless hampers clozapine initiation and treatment^{16,20}.

The presence of lower neutrophil counts in populations with African ancestry has a strong genetic component and has been robustly associated with the Duffy-null polymorphism rs2814778, an African-specific regulatory variant of *Atypical Chemokine Receptor 1 (ACKR1/DARC)*²⁵⁻²⁹. The impact of genetic variants associated with lower neutrophil counts, and in particular the Duffy-null genotype, has not been investigated in the context of clozapine treatment.

In this study, we report the first GWAS of neutrophil levels during treatment with clozapine in individuals with treatment-resistant schizophrenia and robustly inferred African genetic ancestry.

Methods

Sample

Individuals included in this study were from the CLOZUK2 sample, all of whom were prescribed clozapine in the UK with a clinician diagnosis of treatment-resistant schizophrenia. The samples were acquired anonymously in partnership with Leyden Delta (Nijmegen, Netherlands), a company that supplies and monitors clozapine in the UK, as part of the CRESTAR collaborative project (www.crestar-project.eu). The project has received UK National Research Ethics Service approval and was in accordance with the UK Human Tissue Act. All samples were anonymised and linked with blood monitoring data provided from clozapine blood monitoring databases. Full details of the CLOZUK2 sample are provided elsewhere³⁰.

Neutrophil data

The neutrophil count for each individual was defined as the lowest absolute neutrophil count (ANC) on record during clozapine treatment within the blood monitoring database held by Leyden Delta, who retain all historical blood results within their monitoring system. It was not possible to obtain baseline neutrophil counts prior to clozapine

treatment. For clarity, the terms “ANC” and “neutrophil count” are used interchangeably. All results of ANC <1500 cells/mm³, indicating neutropenia and the standard threshold that triggers discontinuation of clozapine in the UK, were confirmed by either a) a consecutive ANC <1500 cells/mm³ or b) two or more results of ANC <2000 cells/mm³ before or after the index result. We excluded individuals who in the opinion of their treating clinician had an alternative explanation for neutropenia such as concomitant immunosuppressive medication (n = 4).

Genotype quality control and imputation

The CLOZUK2 sample was genotyped by deCODE Genetics (Reykjavik, Iceland) on the Illumina HumanOmniExpress-12 chip. PLINK v1.9³¹ was used for genotype quality control following standard protocols³². Maximum per-individual and per-marker missingness were set at 2%, and individuals with inbreeding coefficients (F) higher than 0.2 were removed from the dataset. After this curation process, 7,287 individuals genotyped at 698,442 markers remained in the dataset.

Genotype imputation was performed using the Haplotype Reference Consortium (HRC) panel and the pipeline offered by the Michigan Imputation Server^{33,34}. As this pipeline allowed for imputation of autosomes only, genotype data from the X-chromosome were imputed on the Cardiff University RAVEN cluster³⁵ using the SHAPEIT/IMPUTE2 algorithms³⁶ and a combination of the 1000 Genomes phase 3 (1KGp3) and UK10K reference panels³⁷. Both approaches to genotype imputation have been shown to produce compatible results and to perform similarly in terms of accuracy for variants with minor allele frequencies (MAF) larger than 1%³³. After imputation, 20 million SNPs with INFO scores higher than 0.8 remained in the dataset.

Defining Genetic Ancestry

In order to select a cohort of individuals with African genetic ancestry, we stratified the CLOZUK2 individuals using Ancestry Informative Markers (AIMs), routinely employed in the field of forensic genetics³⁸. The use of AIMs has been shown to be an efficient way of inferring biogeographical ancestry^{39,40}, which reflects the genetic association of an individual to a particular continental or sub-continental population group. As these groups are broadly defined and show relatively large genetic differentiation⁴¹, this approach circumvents the problems associated with several other approximations to genetic ancestry⁴², such as self-reported ethnicity⁴³ or country-of-origin⁴⁴. Our analysis

of AIMs in the CLOZUK2 identified 566 individuals of Sub-Saharan African ancestry, of whom 552 had complete neutrophil count and covariate data, which we term CLOZUK2-AFR. Details on the procedure used to select these individuals are provided in **Supplementary Methods**.

Post-imputation curation of the CLOZUK-AFR genotype data

From this sample, a total of 13.5 million SNPs were taken forward for analysis after applying a MAF filter of 1% and a Hardy-Weinberg Equilibrium (HWE) filter of $p \leq 1 \times 10^{-6}$. HWE tests were carried out using the exact “mid-p” test implemented in the “HardyWeinberg” R package⁴⁵, as this test is valid for both autosomal and sex-linked markers. Relatedness was assessed using the PC-Relate approach, which identified 18 pairs of relatives ($\hat{\pi} \geq 0.2$). For analyses sensitive to confounding by including related individuals, such as contingency table tests, we excluded one random member of each of these pairs. Otherwise, all individuals were included.

Association analysis of neutrophil count

Imputed data from the CLOZUK2-AFR individuals was analysed using the linear mixed model (LMM) implemented in GCTA v1.26⁴⁶, specifically the “leave-one-chromosome-out” procedure⁴⁷. Genotype relatedness matrices, needed to control for population stratification and family structure in LMM frameworks, were calculated directly from the genotyped SNPs. Covariates used in the analyses included gender, age (at lowest ANC), age² and total days on clozapine treatment. PLINK v1.9⁴⁸ was used to identify index SNPs in approximate linkage equilibrium ($r^2=0.1$) using the LD-clumping procedure, with a p-value cut-off of 10^{-4} and a distance cut-off of 3000 kb. Conditional analyses to further identify independent index SNPs were carried out using the GCTA-COJO procedure⁴⁹.

Imputation and analysis of human leukocyte antigen (HLA) alleles

Previous research into clozapine-associated neutropenia has highlighted polymorphisms of the HLA system as drivers of adverse drug reactions in schizophrenia^{22,23,50}. In order to investigate these, we imputed HLA classical alleles using the software HIBAG v1.12⁵¹ (see **Supplementary Methods** for further details). Association testing of the HLA classical alleles used linear regression of dosages weighted by imputation probabilities, following Levin et al. 2015⁵². Covariates employed matched the GWAS described before, though

we added the first 5 principal components calculated by PC-AiR⁵³ to correct for potential population stratification.

Results

Absolute neutrophil count in African individuals

A GWAS of lowest ANC during treatment with clozapine in a total of 552 individuals of African ancestry (**Figure 1; Supplementary Figure 1**, $\lambda_{GC} = 0.985$) identified six independent SNPs ($r^2 < 0.1$) that were associated at the genome-wide significance level of $p < 5 \times 10^{-8}$ (**Table 1**). Five of those SNPs were in close proximity at a locus (1q23.2) tagging, among others, *ACKR1*, previously called the Duffy Antigen Receptor Complex (*DARC*). The most significantly associated SNP in that locus was rs2814778 ($\beta = -0.86$, $p = 4.21 \times 10^{-21}$), which is a regulatory variant in the *ACKR1* promoter region. None of the other SNPs remained genome-wide significant after an analysis conditional on rs2814778, indicating this SNP is responsible for the association signal of the entire locus (**Supplementary Figure 2**). The other genome-wide significant signal was found on chromosome 9 (9q32), and its index SNP was rs77198048 ($\beta = 0.34$, $P = 8.95 \times 10^{-9}$), an intronic variant (MAF = 1.07%) of the Zinc Finger Protein 618 (*ZNF618*) gene.

rs2814778 genotype effect on neutrophil counts

We examined the effects of the different rs2814778 genotypes on ANC after excluding 18 related individuals who were included in the mixed model analysis, leaving 534 CLOZUK2-AFR in the sample. Of these, 419 individuals were homozygous for the C (African) allele, 106 were heterozygous and 9 homozygous for the T (European) allele. A Mann-Whitney test ($U = 318$, $P = 0.099$) did not show significant ANC differences between CT and TT individuals, supporting previous evidence that the C allele has a recessive effect on neutrophil counts²⁵. Thus, for all further analyses we combined individuals with TC and TT genotypes. **Figure 2A** displays the distribution of neutrophil counts during treatment with clozapine by rs2814778 genotype in our African ancestry sample. Individuals with the CC genotype had a median lowest ANC of 1900/mm³ compared to 2900/mm³ for CT/TT individuals ($U = 9240$, $P = 3.55 \times 10^{-24}$).

Figure 2B is a density plot showing the ANC distribution in different CLOZUK2 ancestry subsets (**Supplementary Methods; Supplementary Table 5**), stratified by rs2814778

genotype. While the difference between CC and CT/TT individuals is clearly shown, the CC neutrophil distributions are similar among Sub-Saharan Africans and North Africans (Kolmogorov-Smirnov test p -value=0.941). Similarly, the neutrophil distributions of CT/TT groups show no difference between Sub-Saharan Africans and all other ancestries (Kolmogorov-Smirnov test p -value=0.234, see **Figure 2B**).

Given these results we then tested explicitly whether the rs2814778 Duffy-null genotype is more informative of ANC than genetic ancestry, using generalised linear modelling (**Supplementary Methods**). A model including genetic ancestry (European or African) and the GWAS covariates explained 8.29% of the variance in ANC. When the rs2814778 genotype was added to this model, the variance explained increased to 10.94%, and genetic ancestry was no longer associated with ANC. The removal of genetic ancestry resulted in a statistically equivalent model (likelihood ratio test p -value=0.794) indicating that the rs2814778 genotype is more informative of ANC than genetic ancestry.

A total of 83 (19.81%) CC individuals had neutropenia during treatment with clozapine ($ANC < 1500/mm^3$) in comparison to 2 (1.74%) individuals with a T allele (**Table 2**). In both the US and UK, thresholds of ANC below which alterations in clozapine monitoring and management are indicated, have been defined based on normative values from European populations. In the UK, ANC results below $2000/mm^3$ mandate closer monitoring and more regular blood testing whereas $ANC < 1500/mm^3$ requires clozapine treatment to be withdrawn. We used Barnard's exact test to estimate the effect size of rs2814778 on crossing these thresholds, given their important clinical implications. CC individuals are much more likely to develop an $ANC < 2000/mm^3$ (OR = 6.84, 95% CI = 4.13-13.67, $P = 2.90 \times 10^{-16}$), and an $ANC < 1500/mm^3$ (OR = 20.36, 95% CI = 5.37-314.28, $P = 3.44 \times 10^{-7}$) than T allele carriers. We could not test genotype-mediated differences at lower ANC thresholds, due to the absence of CT/TT carriers.

rs2814778 and benign ethnic neutropenia

There are regulatory mechanisms in place in the US and UK to lower the neutropenia threshold at which clozapine has to be discontinued for those deemed to have benign ethnic neutropenia (BEN), a hereditary condition characterised by mild, chronic neutropenia⁵⁴⁻⁵⁷. Of the 74 individuals in our sample with a formal diagnosis of BEN provided by a Consultant Haematologist, 72 (97.30%) have the CC genotype for rs2814778 (**Table 2**). Considering the safety and clinical outcomes of the 83 individuals

with a CC genotype and ANC <1500 cells/mm³, a total of 80 were rechallenged with clozapine. Of these, at the time of data collection 75 (93.75%) were still maintained on treatment, 4 (5.0%) had subsequently discontinued, and 1 had died (1.25%, unrelated to ANC).

Association analysis of HLA alleles

Using the HIBAG pipeline, we were able to impute 11 *HLA-DQB1* classical alleles and 21 *HLA-B* classical alleles at MAF > 1% in the CLOZUK2-AFR sample; none were significantly associated with ANC after Bonferroni correction for multiple testing ($P < 0.05/32 = 1.56 \times 10^{-3}$), although the HLA-B*45:01 allele was nominally significant ($P = 4.45 \times 10^{-3}$).

Assessment of previous findings from European populations

We were unable to impute any of the following variants implicated in recent association studies of clozapine-associated neutropenia in European populations due to the risk alleles being absent or very rare in CLOZUK2-AFR; rs149104283 (intronic to transcripts of *SLCO1B3* and *SLCO1B7*)²³, the *HLA-DQB1* candidate SNP rs113332494²³, or the *HLA-DQB1* (126Q) and *HLA-B* (158T) amino acid polymorphisms²².

Discussion

In the first genetic association study of neutrophil counts during clozapine treatment in individuals of African ancestry, we identify two genome-wide significant loci. The most significant association is attributable to rs2814778 ($P = 4.21 \times 10^{-21}$), a regulatory variant in *ACKR1* which has previously been associated with lower neutrophil counts in individuals of African ancestry, and is thought to be causal for BEN. We demonstrate that in those taking clozapine, individuals homozygous for the C allele for rs2814778, also known as the Duffy-null genotype, are substantially more likely (O.R. = 20.36) to be classified as having neutropenia (ANC < 1500 cells/mm³), the threshold at which clozapine must be withdrawn in the absence of BEN diagnosis. We further show that genotype at rs2814778 is a superior predictor of neutropenia than the current procedure for diagnosing BEN via haematological clinical assessment, and in doing so, suggest that genotyping at this locus may have clinical utility as a pharmacogenetic test.

The rs2814778 Duffy-null genotype is rare in most non-African populations⁵⁸ but in those of African ancestry, it has been robustly implicated in white blood cell and neutrophil counts in several large meta-analyses²⁵⁻²⁸. It is also considered to be the cause of BEN⁵⁵, an hereditary condition characterized by low neutrophil counts which occurs in 25-50% of individuals with African or Middle Eastern ancestry⁵⁴⁻⁵⁷. In support of rs2814778 as causal for BEN, over 97% of individuals diagnosed with BEN in our study were homozygous for the C allele. However, for those on clozapine, neither ancestry nor a clinical diagnosis of BEN was as good a predictor of low ANC as rs2814778 genotype. In contrast, the distribution of neutrophil counts closely followed genotype at rs2814778 regardless of ancestry (African, European and South-West Asian) (**Figure 2B**). Given that rs2814778 is the likely cause of BEN and that the former performs better than the latter in predicting low ANC, this study implies that BEN is not adequately diagnosed in individuals treated with clozapine. Indeed this is demonstrated by our finding that only 59% of those with the CC genotype and an ANC between 1000 and 1500 cells/mm³ had a clinical BEN diagnosis and were thus able to re-initiate clozapine. Indirect evidence also supports the hypothesis that BEN is under-diagnosed; BEN had a much lower frequency (14%) in our sample than expected from its prevalence of 25-50% in healthy populations of African ancestry⁵⁴⁻⁵⁷. Furthermore, the rates of BEN diagnosis in this sample will be overestimated given its cross-sectional nature (at the point of sample collection), which enriches for those that have been re-challenged with clozapine. Others have similarly noted under-representation of BEN in smaller samples of people of African ancestry taking clozapine¹⁶.

The Duffy-null rs2814778 (C) allele disrupts an erythroid transcription factor GATA-1 binding site in the promoter of *ACKR1* and as a result, the erythrocytes of homozygote rs2814778 carriers do not express ACKR1 protein⁵⁹. Recent experimental work in mice has shown that ACKR1 deficiencies during early haematopoiesis do not result in reduced production of neutrophils, rather neutrophils exhibit altered phenotypic characteristics that result in their preferential loss from blood by egress into tissues, particularly via migration to the spleen, thus causing neutropenia⁶⁰. Importantly there is good evidence that BEN does not lead to increased rates of infection or clozapine-associated agranulocytosis^{19,20,61}. In light of this, for patients with a diagnosis of BEN, clozapine monitoring thresholds in the UK are reduced to ANC > 1500 cells/mm³ and ANC < 1000 cells/mm³ for initiation and discontinuation, respectively. The recently implemented

Clozapine Risk Evaluation and Mitigation Strategy (REMS) program in the US also allocates BEN patients separate monitoring thresholds of ANC > 1000 cells/mm³ for initiation and ANC < 500 cells/mm³ for discontinuation.

Our study supports the safety of separate thresholds for those with BEN; 94% of rs2814778 C homozygotes with ANC < 1500 cells/mm³ were successfully maintained on clozapine treatment after reinstatement. However, in clinical practice, the process of diagnosing BEN is challenging, particularly for those who may be acutely psychotic at the point of clozapine initiation or as a result of clozapine withdrawal. In the UK and the US, a BEN diagnosis is made by a specialist in haematology after assessing the individual's ancestral background, drug history, and the presence of stable low neutrophil counts in the absence of infection. All this necessitates referral by psychiatrists, attendance at haematology outpatient clinics, further blood sampling and review. In light of these practical considerations, it is perhaps unsurprising that only a minority of those who have treatment-resistant schizophrenia and who are eligible for a BEN diagnosis actually receive it, and then go on to have appropriate management with clozapine¹⁶.

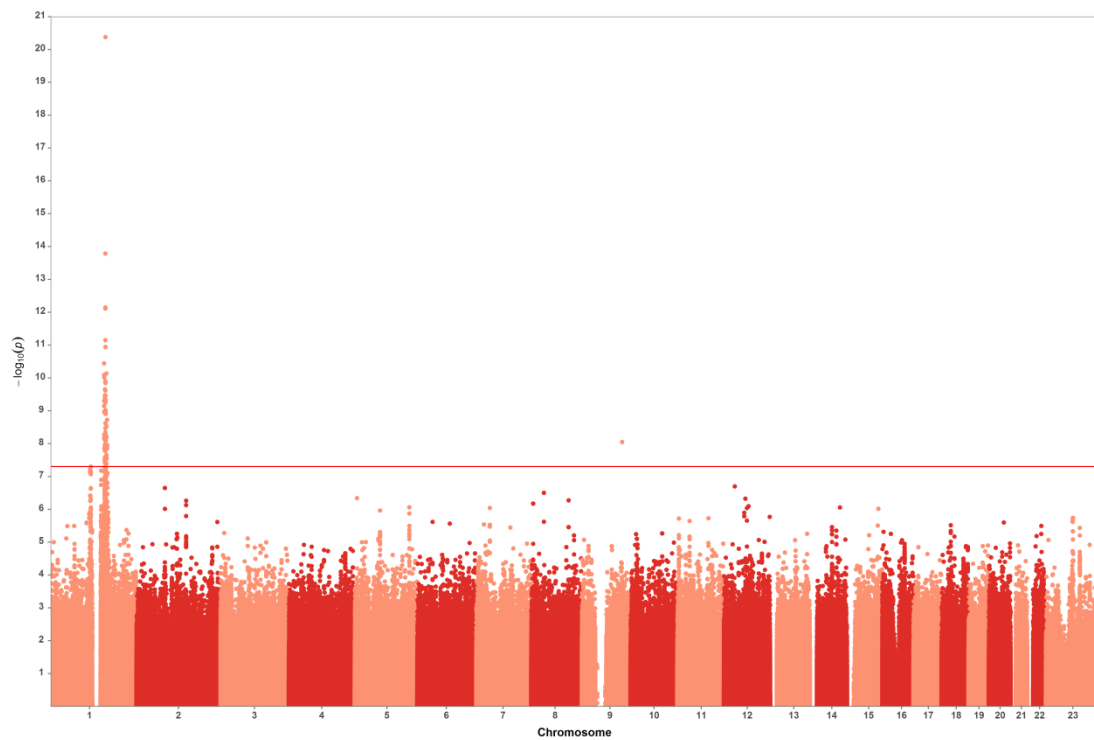
Our results indicate that genotyping rs2814778 offers a simple but sensitive alternative strategy for the diagnosis of BEN. In the context of clozapine treatment, individuals who are homozygous for the C allele and who do not show signs of compromised immune function could have revised neutrophil thresholds in line with current BEN monitoring procedures. This ability to prospectively lower acceptable neutrophil thresholds would address the underutilisation of clozapine in those of African ancestry in the US^{14,15} and UK¹⁶ by (i) enabling more of those suitable for clozapine to start the medication given a lower baseline threshold (ii) avoiding disruption of treatment for those who discontinue clozapine and may or may not under current arrangements be subsequently diagnosed with BEN. In addition to identifying BEN, rs2814778 could also avoid misclassification; the two individuals that had a BEN diagnosis but did not have the Duffy-null genotype in this study have likely been diagnosed incorrectly and could therefore be at increased risk of agranulocytosis. The potential applications extend beyond those of self-reported African ancestry given rs2814778 occurs at non trivial rates in other in which malaria has been historically endemic, including some parts of the Middle East, South West Asia and Oceania⁵⁸.

It is important to note that the Duffy-null genotype is the main causal factor for the Duffy-null phenotype (classically termed “Fy(a-b-)”)⁵⁸. Recent studies have shown that direct genotyping is the best available method to identify Duffy-null individuals in the context of neutropenia^{62,63}, since in the isolated handful of reported cases with Duffy-null phenotype in the absence of the rs2814778 mutation, neutropenia is not observed⁶⁴. A further challenge to using Duffy-null phenotyping arises from other *ACKR1* mutations causing weak antigen expression which can mimic the Duffy-null phenotype causing serological ambiguity, resulting in misclassification of up to 3.5% of individuals depending on population^{62,65,66}. For this reason, we argue that genotyping of rs2814778 would outperform the serological typing of the Duffy-null phenotype.

The second genome-wide significant finding corresponds to an intronic variant in the *ZNF618* gene, which has been characterised recently as a contributor to methylation and chromatin binding of epigenetic regulators⁶⁷. However, the associated locus on chromosome 9 contains a number of other genes and thus, based on the evidence from this study alone, a possible causal link with neutrophil counts or clozapine-associated effects cannot be inferred.

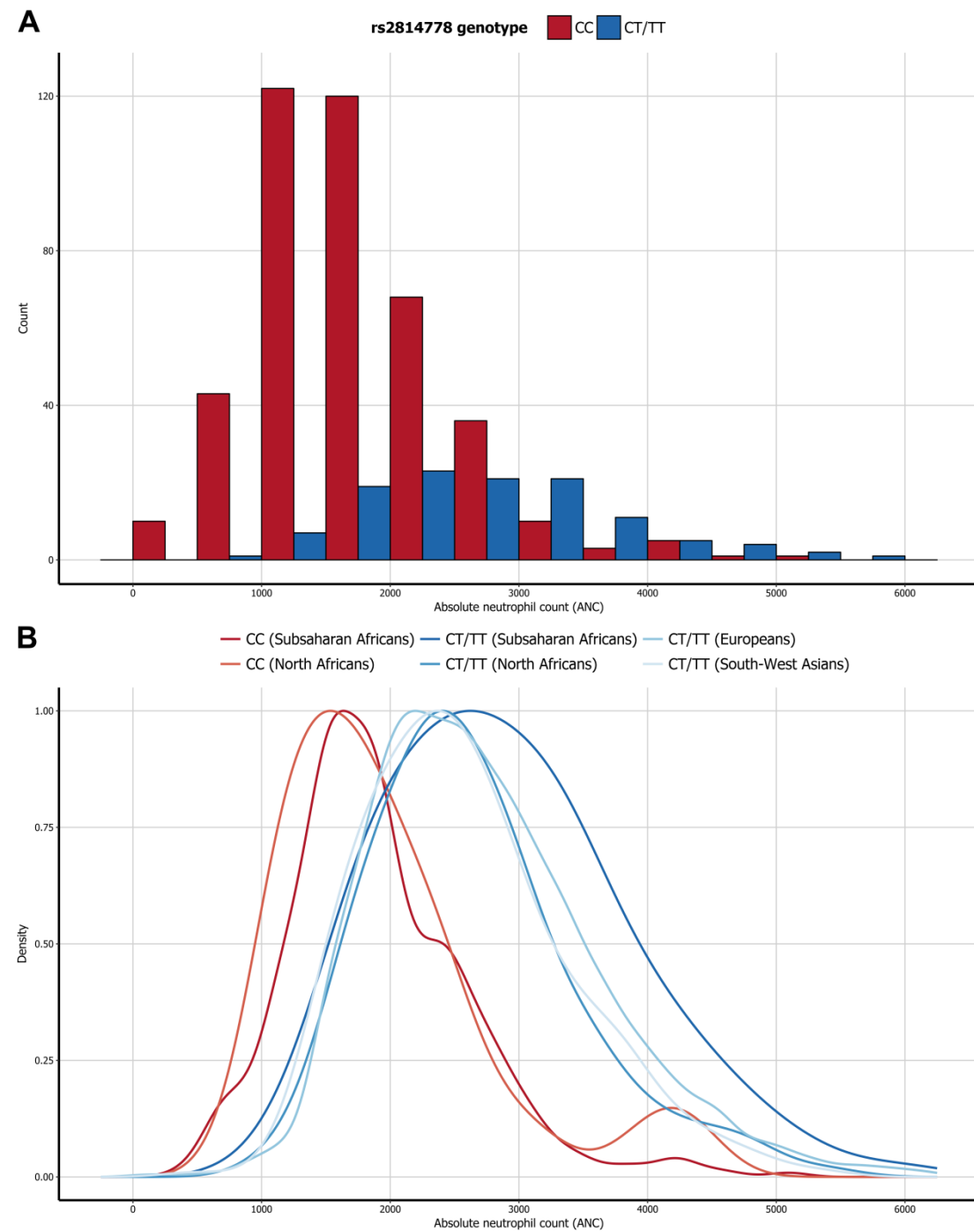
In summary, we provide novel insights into the genetic architecture of neutrophil counts during clozapine treatment in individuals with African genetic ancestry. We demonstrate strong association with rs2814778, a regulatory variant in the *ACKR1* gene that has also been described as the genetic basis for BEN. We suggest that rs2814778 genotyping offers an as yet rare opportunity for personalised medicine in psychiatry. Although we recognise further research is needed to establish the acceptability, uptake, clinical utility, and the practical outcomes and cost-benefits of such a test, rs2814778 genotyping has the potential to assist decision making in the initiation and on-going management of clozapine treatment. Moreover the test presents an opportunity to increase uptake of clozapine in those of African ancestry, for whom access to the only effective medication for treatment-resistant schizophrenia is currently often denied.

Figure 1



Manhattan plot of the lowest ANC GWAS in the CLOZUK2-AFR sample. The genome-wide significant peak at chromosome 1 corresponds to the *ACKR1* locus mentioned in the text.

Figure 2



A: Histogram of lowest neutrophil count in the CLOZUK2-AFR sample, stratified by rs2814778 genotype. B: Scaled density plots of ANC in the different CLOZUK2 biogeographical ancestry subsets, stratified by rs2814778 genotype.

Table 1

Genome-wide significant SNPs from the ANC GWAS in the CLOZUK2-AFR sample.

SNP	CHR	BP	A1	A1 Frequency	Beta	s.e.	P-value
rs2814778	1	159174683	C	88.38%	-0.856	0.091	4.21 x10 ⁻²¹
rs4971072	1	155273869	G	92.25%	-0.700	0.106	3.61 x10 ⁻¹¹
rs260913	1	163968414	C	4.45%	0.788	0.131	1.89 x10 ⁻⁹
rs12128479	1	162268123	G	1.63%	1.205	0.203	2.98 x10 ⁻⁹
rs77198048	9	116789254	T	1.07%	1.625	0.282	8.95 x10 ⁻⁹
rs12143237	1	162480145	A	2.90%	0.877	0.153	1.11 x10 ⁻⁸

Table 2

ANC and BEN diagnosis in CLOZUK2-AFR, stratified by rs2814778 genotype.

	CC		TC/TT	
	N (% total CC)	N BEN (%)	N (% total TC/TT)	N BEN (%)
ANC < 0.5	1 (0.24%)	1 (100.00%)	0 (0.00%)	-
0.5 ≥ ANC < 1	19 (4.53%)	14 (73.68%)	0 (0.00%)	-
1 ≥ ANC < 1.5	63 (15.04%)	37 (58.73%)	2 (1.74%)	1 (50.00%)
1.5 ≥ ANC < 2	149 (35.56%)	17 (11.40%)	16 (13.91%)	0 (0.00%)
ANC ≥ 2	187 (44.63%)	3 (1.60%)	97 (84.35%)	1 (1.03%)
Total	419 (100.00%)	72 (17.18%)	115 (100.00%)	2 (1.73%)

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Acknowledgements

This project was supported by Medical Research Council (MRC) Centre (MR/L010305/1), Program (G0800509), and Project (MR/L011794/1) grants to Cardiff University. The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 279227 (CRESTAR Consortium; <http://www.crestar-project.eu/>). This publication reflects only the authors' views and the European Union is not liable for any use that may be made of the information contained therein.

We acknowledge Leyden Delta and Magna Laboratories, UK for supporting the CLOZUK2 sample collection, anonymisation and data preparation (Andy Walker and Anouschka Colson). We acknowledge deCODE genetics (Hreinn Stefansson and colleagues) for genotyping of the CLOZUK2 sample. We acknowledge the MRC Centre laboratory staff (particularly Lucinda Hopkins, Lesley Bates, and Catherine Bresner) at Cardiff University, for laboratory sample management and Wayne Lawrence and Mark Einon, at Cardiff University, for support with the use and setup of computational infrastructures.

Competing Financial Interests

D. A. C. is a full-time employee and stockholder of Eli Lilly and Company. M. H., J. J. & K. J. are full-time employees of Leyden Delta B.V. The remaining authors declare no conflicts of interest.